# COMPOSITION AND APPARENT DIGESTIBILITY OF THE CARBOHYDRATE AND OTHER CONSTITUENTS OF PEA AND LIMA-BEAN VINES WHEN FED TO SHEEP 1

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# INTRODUCTION

Pea vines and lima-bean vines 3 obtained as byproducts from pea and lima-bean canneries are annually available in large quantities and constitute a potential source of feed for farm animals. Pea vines have been used as a fodder for farm animals for many years. This material is relatively high in protein and cattle eat it readily. Lima-bean vines are available in considerably smaller quantities, and

they have not been used to any great extent as stock feed.

The hemicelluloses constitute an important part of the undetermined fraction of hays and coarse fodders, conventionally designated as "nitrogen-free extract," and the extent to which they contribute to the maintenance of the animal is a matter of considerable importance. Pea and lima-bean vines are known to be especially rich in hemicelluloses, and these materials are therefore particularly suited for a study of the apparent digestibility of these carbohydrate complexes.

This paper reports a study of the composition and apparent digestibility of the carbohydrate and other constituents of pea and lima-bean vines preserved by artificial dehydration, with particular attention to the hemicelluloses and related constituents.

used as the experimental animals.

## REVIEW OF LITERATURE

Rupel, Roche, and Bohstedt (21)4 in 1931 reported that artificially dried pea-vine hay, when fed to cows, was equal in feeding value to

good quality chopped alfalfa hay.

Tretsven (17) in 1936 reported two trials with pea-vine silage as a feed for dairy heifers. For milk production and growth, the pea-vine silage was worth approximately one-third the value of good quality alfalfa hay. When fed with suitable precaution, pea-vine silage had no influence on the flavor of the milk produced.

Johnson and Peterson (7) in 1940 reported the results of a study of the milk production and the vitamin A potency of the butterfat of a herd of dairy cows alternately receiving a ration that included

Italic numbers in parentheses refer to Literature Cited, p. 186.

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³ The terms "pea vines" and "lima-bean vines" as used in this paper include the empty pods as well as

25 pounds of pea-vine silage preserved with phosphoric acid and a dry ration in which alfalfa hay replaced the silage. The milk produced on the silage ration was markedly higher in carotene and vitamin A than that produced on the dry ration and the level of milk

production was also more satisfactory.

Woodman and Evans (22) reported the results of digestion trials with sheep fed artificially dehydrated pea-pod meal and broad-bean-pod meal. On a dry-matter basis the pea-pod meal and bean-pod meal contained, respectively, 10.82 and 11.17 percent digestible crude protein; 0.94 and 0.62 percent digestible ether extract; 46.12 and 44.81 percent digestible nitrogen-free extract; and 10.63 and 10.37 percent digestible crude fiber. Woodman and Evans concluded that both products can be considered as desirable constituents in the rations of sheep.

Practically all previous investigators of the nutritive value of hemicelluloses have based their conclusions as to the apparent digestibility of these carbohydrates on the percentages of the total furfural afforded by the food consumed and the feces eliminated. Any method for the determination of hemicelluloses based on the assumption that only these carbohydrate complexes afford furfural when boiled with mineral acids is likely to lead to a rather serious error, and therefore much of the data on the digestibility of hemicelluloses must be accepted with considerable reserve. A critical résumé of the methods used for the quantitative estimation of hemicelluloses is presented elsewhere in this paper.

Fraps (3) found that the total pentosans of legumes were on an average digested better than the pentosans of nonlegumes. He also noted that the pentosans soluble in 0.02 N acid and alkali were

better digested than the remaining pentosans.

Wille (20) in 1918 reported observations indicating the presence of a hemicellulose-splitting enzyme in the parotid glands and in the gall of cattle and swine, and in the stomach, pancreas, and small intestine of swine. Wheat bran, slices of the endosperm of lupine seeds, and cross sections of the rhizomes of *Molinia caerulea* were used as sources of hemicellulose materials.

Rippel (15) in 1921 reported the presence of enzymes in the seeds of Lupinus angustifolius, Galium aparine, and Asparagus officinalis capable of digesting the hemicelluloses of these seeds, and he took the view that the digestion effects observed in Wille's experiments were produced, in part at least, by the plant enzymes introduced

with the hemicellulose material.

Shimizu (16) in 1921 reported results of digestion experiments conducted in vitro with extracts of macerated intestine and pancreas of dogs and rabbits. No monosaccharide could be found in the reaction mixture either by the phenylhydrazine or the Fehling reduction test and Shimizu concluded that there were no enzymes in such preparations capable of hydrolyzing hemicelluloses.

According to Manville, Bradway, and McMinis (13), galacturonic acid plays a role similar to that of glucuronic acid in the detoxication mechanism of the animal body. They, therefore, consider that foods containing pectin and hemicelluloses have a value separate and

distinct from caloric considerations.

## EXPERIMENTAL MATERIAL AND PROCEDURE

#### METHODS OF ANALYSIS

The pea and lima-bean vines used in this investigation were obtained from a cannery in Pennsylvania. As soon as the vines came off the viners, they were hauled by truck about 30 miles and dried in a commercial drier. The dry material was then shipped to the Beltsville Research Center at Beltsville, Md., where it was stored in a dry barn until used. Some of the lima-bean vines were moldy, but it is not known at what stage the mold developed.

DETERMINATION OF AMMONIUM OXALATE EXTRACTIVES AND FURFURAL-YIELDING CONSTITUENTS

There is at present no satisfactory method for the quantitative estimation of hemicelluloses. The method most commonly used by agricultural chemists for the determination of hemicelluloses, generally called pentosans, is based on the fact that these substances when boiled with mineral acids yield furfural. This method, which was developed by Tollens and his school, is an empirical one. According to Kröber's (9) procedure, the furfural is precipitated with phloroglucinol and from the weight of phloroglucide obtained, the weight

of pentosans is calculated.

While the method is quite satisfactory for the determination of pure pentose sugars or reasonably pure xylan or araban, there are objections to it when applied to the determination of hemicelluloses. In the first place, when a plant material is distilled with mineral acids, the furfural obtained is derived not only from the hemicelluloses, but also from pectic substances, so that the yield of furfural is frequently higher than would be the case if only hemicelluloses were present. Moreover, there is considerable difference in the percentage of furfural obtained from various hemicelluloses because of the variable composition of these carbohydrate complexes, so that it is impossible to correlate the percentage of furfural found with the percentage of hemicelluloses in the plant material under examination.

Accordingly, in the present investigation no attempt was made to determine the hemicelluloses as such. Instead, the sample under investigation was first freed from pectic substances by extraction with a hot 0.5-percent aqueous ammonium oxalate solution and then the percentage of furfural in the residual material was determined. The result thus obtained was calculated as the percentage furfural in the original unextracted sample and is recorded in tables 2, 3, and 4 under the heading, "Furfural-yielding constituents reported as furfural." It is realized, of course, that the percentage of furfural thus found is only an approximate indication of the percentage of hemicellulose in the sample, but since there is no satisfactory method for the direct determination of the carbohydrate complexes, the procedure followed here is believed to be the best available. The determination was carried out as follows:

To a 2-gm. sample contained in a 250-ml. centrifuge bottle, 200 ml. of a 0.5-percent aqueous ammonium oxalate solution (previously heated to 90° C.) was added, and the mixture was heated in a water bath maintained at 85° C. for 1 hour. From time to time the reaction mixture was stirred with a glass rod. At the end of the 1-hour period,

the reaction mixture was centrifuged and the clear supernatant solution was poured through a weighed sintered-glass crucible (porosity D). To the residue in the centrifuge bottle, 200 ml. of a 0.5-percent aqueous ammonium oxalate solution (previously heated to 90° C.) was added, and the mixture was heated again for 1 hour in the water bath maintained at 85° C. as previously described. The reaction mixture was centrifuged and the supernatant solution was filtered through the weighed sintered-glass crucible. This operation was repeated twice, making a total of four extractions. It was found experimentally that practically all the pectic substances were removed in the first two extractions. The residual material was then transferred to the weighed sintered-glass crucible, thoroughly washed with hot water, dried at 105° C., and weighed. The result was calculated on the basis of the original moisture-free material and is recorded in tables 2, 3, and 4 under the heading "Ammonium oxalate extractives."

The percentage of furfural in the residual material obtained in the above-described extraction with 0.5-percent aqueous ammonium oxalate solution was determined by the Tollens-Kröber procedure (1). The result obtained was calculated on the basis of the original moisture-free material and is reported in tables 2, 3, and 4 under "Furfural-

yielding constituents reported as furfural."

# CRUDE CELLULOSE

Crude cellulose was determined by the method of Kürschner and Hanak (10).

# DETERMINATION OF PECTIC SUBSTANCES

For the determination of pectic substances, the sample (5 gm.) was first exhaustively extracted with a hot 0.5-percent aqueous ammonium oxalate solution, the procedure previously described in connection with the determination of ammonium oxalate extractives being followed. To the filtered ammonium oxalate extract 3.5 volumes of 95-percent ethanol containing 10 ml. of concentrated hydrochloric acid per liter were added, and the mixture was allowed to stand overnight. It was then transferred portionwise to a 250-ml. centrifuge bottle, the mixture was centrifuged, and the clear supernatant solution was drawn off and discarded. The crude pectic material was shaken with 80-percent ethanol containing 10 ml. of concentrated hydrochloric acid per liter, centrifuged, and the clear solution was drained off and discarded. This operation was continued until the alcoholic washings no longer gave a test for oxalates or oxalic acid. To the pectic material in the centrifuge bottle, 40 to 50 ml. of water and 2 ml. of concentrated ammonium hydroxide solution were added, the mixture was stirred until the pectic substance was completely dissolved, and the solution was then transferred to a beaker. This solution was heated to boiling and filtered through a Hirsch type sintered-glass funnel of fine porosity. The centrifuge bottle was washed twice with 40 to 50 ml. of hot water and the washings were also passed through the sintered-glass funnel. A bell jar arrangement was used for filtering and for collecting the filtrate and washings. The combined filtrate and washings, after cooling to room temperature, were treated with 100 ml. of 0.4 percent of sodium hydroxide solution and were then allowed to

stand overnight. To this 50 ml. of N (approximately) acetic acid was added, together with an equal volume of a molar solution of calcium chloride, and the whole was boiled for 10 minutes. It was then filtered through a weighed soft filter paper (C. S. and S. No. 597) and the precipitate was washed with hot water until it was free of chlorides. It was dried at 105° C., weighed, and the result was calculated as percent of calcium pectate, in the moisture-free plant material.

All other analytical data recorded in this paper were obtained by the methods of the Association of Official Agricultural Chemists (1).

#### FEEDING EXPERIMENTS

For the feeding trials, conducted during the winter and spring of 1942, five Hampshire ewes were used; three were yearlings and two were 2-year-olds. Two ewes were used for each feed in trial 1. the conclusion of this trial the feeds were reversed for each animal, and after a suitable preliminary period, a second trial was made. However, one pregnant animal (19U) that was used in the first trial (table 3) was replaced for the second trial (table 4). The animals were gradually accustomed to the rations to be used during the trials. For the first 8 days they were fed a hay mixture consisting of one part of pea or lima-been vines and one part of alfalfa hay. were also fed a grain mixture of one-half pound daily at the start, but this quantity was gradually decreased to none by the fourteenth day. The animals were then placed on a 13-day preliminary period, on the eighth day of which they were placed in the metabolism cages. The animal that replaced 19U was also gradually accustomed to the feed and had a preliminary period like the others.

During the preliminary period it was found that the sheep ate the pea or lima-bean vines more readily when they were dampened. feed for each animal was weighed daily, and from the same feed source a daily portion was saved for a 10-day composite sample for analysis. The amount of feed given was so adjusted that the animals would have enough to eat and still there would be no excessive accumulation of rejected feed. The refused feed was collected once daily, and at the end of each 10-day trial it was weighed, dried if necessary, weighed again, ground, and sampled for chemical analysis. The pea vines were rather fine, dry, and dusty; the lima-bean vines were coarse and stemmy as well as dry and dusty. Moldy material was discarded.

Metal metabolism cages were used. The feces were retained on a fine copper screen and on alternate days they were collected from the The feces were kept in a covered can, toluene being used as a preservative, and were stored in a refrigerator. At the end of each collection period, the total quantity of feces from each animal was spread out thinly on large sheets of paper, dried in a current of air at 40° C., ground, weighed, and a sample was removed for analysis. The feces dried within 48 hours without molding.

The urine was collected daily from the container kept under each metabolism cage and stored in large glass bottles. It was kept acid at all times by adding 1 ml. of a 10-percent acetic acid solution for each liter of urine collected. The final volume of urine was measured, mixed, and an aliquot was removed for analysis.

In all experiments the animals had unrestricted access to water.

# EXPERIMENTAL RESULTS

The results obtained are recorded in tables 1 to 4.

Table 1.—Gain or loss in weight and nitrogen retained by sheep fed on pea and lima-bean vines

Fed pe	ea vines		Fed lima-	bean vines	
Sheep No.	Gain or loss in weight	Nitrogen retained	Sheep No.	Gain or loss in weight	Nitrogen retained
6V 1	Kilograms +1.0 +.5 +1.6 2	Grams 50. 4 56. 9 76. 4 69. 1	6V 17U 18V 19U	Kilograms +0.3 8 +.4 -1.7	Grams 42. 3 30. 2 35. 4 20. 1
Mean	+.7	63. 2		4	32. 0

<sup>&</sup>lt;sup>1</sup> V = Yearling sheep. <sup>2</sup> U = 2-year-old sheep.

12.42

11.28

7.32

Table 2.—Percentage composition of the samples of pea and lima-bean vines that were fed and refused

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Feed	Ash	Crude protein	True protein	Fat	Crude fiber	Nitro- gen-free extract	Ammo- nium oxalate extrac- tives	Fur- fural- yield- ing constitu- ents re- ported as fur- fural	Crude cellu- lose (ash- free)	Pectic sub- stances (as Ca pectate)
Pea vines: Fed Refused	Percent 12. 81 9. 57	Percent 16.44 10.70	Percent 11. 11 6. 82	Percent 2. 03 1. 28	Percent 23. 63 37. 55	Percent 45. 09 40. 90	Percent 39. 59 32. 39	Percent 6. 45 8. 90	Percent 23. 85 32. 92	Percent 4. 28 3. 33
Lima-bean vines: Fed	11.64	12, 52	8, 17	1, 62	29. 33	44. 89	34. 28	7. 63	30. 38	5. 27

[Calculated on moisture-free basis]

Table 1 shows the gain or loss in weight of and the nitrogen retention by the sheep used in the feeding trials. In the feeding trials with pea vines all the animals, except one, not only maintained their weight, but actually gained weight. The nitrogen retained ranged from 50.4 to 76.4 gm. The animals fed lima-bean vines, on the other hand, lost weight in two feeding trials, and barely maintained their weight in the other two. The nitrogen retained was considerably less in the feeding trials with lima-bean vines than in those with pea vines.

1. 55

32. 26

42.49

30.93

7.60

31.38

4.28

Table 2 shows the average chemical composition of the forage fed and of the feed refused. The percentage of crude protein in the pea vines was greater than in the lima-bean vines. In both materials, as was to be expected, the crude protein and pectic substances were lower, while the crude fiber and crude cellulose were higher, in the refused feed than in the feed offered. The nitrogen-free extract and the ammonium oxalate extractives in both materials were higher in the feed offered, however, than in the feed refused.

In tables 3 and 4 data are given on the dry matter and nutrients ingested, voided, and digested, and the mean apparent digestibility of the various constituents of pea and lima-bean vines, respectively. These tables show that the apparent digestibility of the dry matter

Table 3.—Dry matter and nutrients of lima-bean vines as determined from the material offered and refused and in that ingested, voided, and digested by sheep

[Weight in gram

Pectic sub- stances (as Ca pectate)	710.4 164.4 546.0	295.3	886.4 131.6 754.8	886.4 287.3 599.1	
Crude cellulose (ashfree)	704.04 1,404.8 3,2890.2 2,599.2 7,702.6 7,75.8	2, 894. 3 698. 4 2, 195. 9 75. 9	4, 496. 0 1, 029. 8 3, 466. 2 1, 048. 8 2, 417. 4 69. 7	4, 496. 0 2, 068. 7 2, 427. 3 534. 1 1, 893. 2 78. 0	74.8
Furfural- yielding constituents reported as furfural	1, 166.4 328.3 838.1 337.1 501.0 59.8	1, 100.1 133.5 732.9 282.2 450.7 61.5	1, 144. 0 251. 0 893. 0 381. 1 511. 9 57. 3	1, 144. C 521. 5 622. 5 225. 6 396. 9 63. 8	60.6
Ammoni- um oxalate extractives	171. 323. 323. 72. 79.	1, 808.5 3, 362.7 764.3 2, 598.4 77.3	5, 211. 2 956. 5 4, 254. 7 055. 6 3, 299. 1 77. 5	5, 211. 2 2, 231. 0 2, 980. 2 645. 8 2, 334. 4 78. 3	78.0
Nitrogen- free extract	692. 821. 116. 775.	2, 506. 1 4, 186. 7 4, 186. 7 984. 4 3, 202. 3 76. 5	6, 897. 6 1, 359. 9 5, 537. 7 1, 289. 0 4, 248. 7	6, 897. 6 2, 939. 3 3, 958. 3 812. 2 3, 146. 1 79. 5	77.4
Crude fat Crude fiber	547. 466. 080. 170. 909. 62.	1, 735.2 2, 812.0 1, 034.8 1, 777.2 63.2	4, 336. 0 1, 087. 1 3, 248. 9 1, 428. 0 1, 820. 9 56. 0	4, 336. 0 2, 177. 9 2, 158. 1 811. 1 1, 347. 0 62. 4	6.09
Crude fat	244.8 63.4 8 181.4 4 96.6 6 46.7 7	74.7 91.7 153.1 76.9 76.2 49.8	246.4 50.1 196.3 108.6 87.7 44.7	246.4 110.6 135.8 65.9 69.9 51.5	48.2
True pro- tein	1, 260.8 299.1 3 961.5 438.5 523.0 54.4 4	1, 200. 453. 6 807. 2 896. 0 411. 2 50. 9	1, 214. 4 230. 9 983. 5 486. 0 497. 5 50. 6	1, 214. 4 508. 6 705. 8 340. 1 365. 7 51. 8	51.9
Crude pro- tein	i i i	1, 247. 3 1, 247. 3 536. 0 711. 3 57. 0	1,856.0 351.6 1,504.4 675.1 829.3 55.1	1,856.0 788.7 1,067.3 449.4 617.9 57.9	57.0
Ash	1, 729.6 531.6 1, 198.0 802.6 395.4 33.0	1,723.0 755.7 973.9 741.3 232.6 23.9	1, 796.8 386.0 1, 410.8 918.1 492.7 34.9	1, 796.8 854.8 942.0 570.5 371.5	32.8
Dry matter	16, 000 4, 591 11, 409 7, 386 64. 7	10, 000 6, 886 3, 545 6, 341 64. 1	16, 000 3, 410 12, 590 4, 682 7, 908 62.8.	16,000 7,183 8,817 2,841 5,976 67.8	64.8
Feed for sheep No.—	6V: Offered Offered Ingested Voided Digested Digested TrU:	Refused Ingested Volted Digested (percent).	Offered. Metised. Ingested. Digested. Digested (percent).	Offered.  Retused. Ingested.  Digested.  Digested (percent).	Average digested (percent)

Table 4.—Dry matter and nutrients of pea vines as determined from the material offered and refused and in that ingested, voided, and digested by sheep

[Weight in grams]

Feed for sheep No.—	Dry matter	Ash	Crude pro-	True pro- tein	Crude fat	Crude fiber	Nitrogen- free extract	Ammoni- um oxalate extractives	Furfural- yielding constituents reported as furfural	Crude cel- lulose (ash- free)	Pectic substances (as
eV: Offered Bectused Ingested Voided Digested Digested Digested Digested Digested Noided Noided Noided Refused Refused Noided Refused Refused Digested Digested Voided Voided Voided Refused Voided Voided Refused Voided Voided Refused Voided Voided Noided Voided	16,000 19,006 19,006 19,006 10,000 10,000 10,000 10,000 10,000 10,000 11,400 10,000 11,400 11	1, 952. 0 1, 680.11 1, 285.6 245.3 245.3 245.3 1, 952.0 1, 960.0 1, 960.0 1, 398.9 1, 398.9 1, 398.9 1, 398.0 1, 398.0 1	2, 247, 27, 27, 27, 27, 27, 27, 27, 27, 27, 2			3, 680 0 1, 085 2 1, 085 3 1,	6,835.2 1,137.7 1,137.7 1,236.9 1,396.9 1,397.5 1,397.0 1,397.0 1,097.7 1,097.				708.8 110.5 596.8 156.8 8.66.2 8.66.2 8.66.8 108.8 4.48.8 4.48.8 8.88.8 8.88.8 8.88.8
Average digested (percent)	63.3	24.8	64.7	63.7	53.7	54.9	78.1	78.6	61.2	68.8	

of pea and lima-bean vines was about the same. The apparent digestibility (expressed as percent digested) of the crude protein, true protein, and crude fat of the pea vines was greater than that of the same constituents in lima-bean vines. The apparent digestibility of the nitrogen-free extract, ammonium oxalate extractives, and of the furfural-yielding constituents of both materials was about the same. The apparent digestibility of the crude fiber and crude cellulose in lima-bean vines, however, was greater than that of the same con-

stituents in pea vines. The apparent digestibility of the pectic substances in the two rations is not recorded in tables 3 and 4 because there was some uncertainty as to the extent of the digestibility of the pectic substances of the two plant materials submitted to feeding trials. When the several samples of feces were analyzed for pectic substances, by the method previously described, only small quantities of a dark precipitate were obtained in most cases. This material did not have any of the properties of calcium pectate and the percentage of calcium varied from somewhat under 1 to about 1.5 percent. The material obtained was clearly not calcium pectate, but a mixture of amorphous substances. It would appear that the pectic substances in passage through the digestive tracts of the animals were either completely digested and assimilated or that they were changed or degraded to such an extent that they could no longer be precipitated and determined by the method used. Kertesz (8), as a result of his experiments with dogs and human subjects, concluded that pectins when taken orally are not attacked until they reach the large intestine where they are completely hydrolyzed by bacterial enzymes.

## DISCUSSION

As mentioned earlier in this paper, there is at present no satisfactory method for the quantitative estimation of hemicelluloses. method used in this investigation, a separation was made between the two principal furfural-yielding constituents, namely, the pectic substances and the hemicelluloses. While the figures in the columns headed "Furfural-yielding constituents reported as furfural" in tables 2, 3, and 4 cannot readily be converted into absolute quantities of hemicelluloses, they do nevertheless bear a close and direct relationship to the actual hemicellulose content of the materials under investigation. The results of the feeding trials with pea and lima-bean vines indicate very definitely that a considerable portion of the hemicellulosic material ingested was apparently digested, at least to the extent that there was a definite removal or destruction of the furfural-yielding components, in the hemicellulose complex. It is, of course, not known to what extent these components of the hemicellulose complex were actually utilized by the sheep as sources of energy or for other purposes. Moreover, it must be remembered that in addition to the furfuralyielding components, namely, pentosans and uronic acids, many of the hemicelluloses contain also hexosans and the analytical method used did not and could not measure the extent to which these were utilized by the sheep. In other words, the hemicelluloses in the pea and limabean vines may have been digested to a greater extent than is indicated by the data on the digestibility of the furfural-yielding constituents in tables 3 and 4.

# SUMMARY

Feeding trials were conducted with sheep fed exclusively diets of either pea or lima-bean vines.

The animals fed an exclusive diet of pea vines not only generally

maintained their weight, but most gained some weight.

The animals fed an exclusive diet of lima-bean vines lost weight in two feeding trials while in two others they barely maintained their

The respective mean percentages of the apparent digestibility of the various constituents of pea and lima-bean vines were as follows: Crude protein, 64.7 and 57.0 percent; true protein, 63.7 and 51.9 percent; crude fat, 53.7 and 48.2 percent; crude fiber, 54.9 and 60.9 percent; nitrogen-free extract, 78.1 and 77.4 percent; ammonium oxalate extractives, 78.6 and 78.0 percent; furfural-yielding constituents, 61.2 and 60.6 percent; and crude cellulose, 68.8 and 74.8

The pectic substances of pea and lima-bean vines in passage through the digestive tracts of sheep were either completely digested or were changed or degraded to such an extent that they could no longer be

determined in the feces by the calcium pectate method.

A considerable portion of the hemicellulosic complexes of the pea and lima-bean vines were apparently digested by the sheep, at least to the extent that they suffered a definite removal or destruction of the furfural-yielding components.

In these trials pea vines were found to be a satisfactory forage feed for sheep, but lima-bean vines were inferior to pea vines as an exclusive

diet for sheep.

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